# Star-crossed Side Effects: Evaluating the Interplay between Toxicity and Efficacy in the Combination Treatment of Trabectedin with HSV1716 in Murine Osteosarcoma

Undergraduate Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation "with Honors Research Distinction in Microbiology" in the undergraduate colleges of The Ohio State University

by
Mackenzie Deighen

The Ohio State University
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Project Advisor: Dr. Timothy P. Cripe, MD, PhD, Department of Pediatrics

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**Abstract**

Pediatric patients with solid tumors have unacceptably low cure rates, and immunotherapy, such as oncolytic virotherapy, provides great promise for treatment. However, there is a lack of knowledge regarding the immunosuppressive microenvironment of solid tumors, and how this microenvironment affects the efficacy of virotherapy and other cancer immunotherapies. Tumor-Associated Macrophages, TAMs, specifically, M-2 like macrophages, and Myeloid Derived Suppressor Cells, MDSCs, are two key targets for improving the efficacy of immunotherapies as both play roles in immunosuppression. As such, it is suspected that a combined treatment that both reduces myelolytic cells in the microenvironment while stimulating a pro-inflammatory response will increase cytotoxic immune infiltration in the tumor microenvironment. We have previously demonstrated that trabectedin, an FDA-approved chemotherapy drug, in combination with oncolytic HSV, oHSV, has shown to stimulate tumor regression in two Ewing sarcoma Patient Derived Xenograft, PDX, models[10]. Our proposed mechanism suggests that trabectedin targets tumor-associated M2-like macrophages, and both depletes their levels while also polarizing remaining macrophages to an M1-like macrophage, which can perform phagocytosis against the tumor. While two Ewing sarcoma PDX models have demonstrated tumor regression in response to this combined myelolytic-virotherapy, it is unknown if these effects are generalizable to other non-Ewing sarcoma pediatric models or syngeneic models. This thesis explores the use of a combination treatment of the oncolytic virus, HSV1716, with the chemotherapy agent, trabectedin, in murine osteosarcoma. Specifically, this thesis focuses on the interplay between the toxicity and efficacy of the combination therapy of HSV1716 and trabectedin.

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## Introduction and Background

Current treatments for pediatric cancer solid tumors include surgery, radiation, and chemotherapy. However, suboptimal cure rates, especially in cases of malignant or recurrent disease, require that new treatment options be made available. Additionally, for children who survive adolescence and into adulthood, the long-term side-effects of current treatments may limit quality of life. Immunotherapy, thought to produce fewer long-term side effects than current treatments, provides a promising field for the development of new treatment options for pediatric patients[1]. Immunotherapy enhances the immune system's ability to detect and eliminate cancer. Immunotherapy agents include antibody therapy, adoptive therapy, checkpoint inhibitors, and oncolytic viruses[1].

Solid tumors are situated within a complex environment of infiltrating stromal and immune cells as well as extracellular components, such as cytokines and growth factors, that promote an immunosuppressive environment[2,3]. The tumor microenvironment varies for each tumor model, however, myeloid-derived suppressor cells, MDSCs, and tumor-associated macrophages, TAMs, are prominent infiltrating cells[2]. TAMs are plastic cells which allow them to change their phenotypic expression in response to cell signaling. M1-like macrophages are activated by interferon gamma (IFNγ) and display anti-tumor effects by releasing pro-inflammatory cytokines to recruit other immune cells to the microenvironment and by direct phagocytosis of the tumor[2,4]. However, most TAMs within the tumor microenvironment are polarized to the M2- like phenotype by interleukin (IL)-4, IL-13, IL-10, and colony-stimulating factor (CSF) 1[2]. The M2-like macrophage is associated with a protumorigenic response by secreting factors that promote proliferation, immunosuppression, and angiogenesis such as vascular endothelial growth factor A (VEGF-A) and transforming growth factor β (TGFβ)[2,4].

TAMs are abundant in pediatric solid tumors, and high TAM infiltrate in the tumor microenvironment is associated with poor prognosis[2,5]. As such, there is a need for therapies that both target the tumor and the tumor microenvironment.

Oncolytic viruses selectively infect and kill cancer cells through either direct lysis or by promoting an antitumor immune response[1,6]. Currently, the only FDA-approved oncolytic virus is talimogene laherparepvec (T-VEC), an attenuated herpes simplex type-1 (HSV-1) for the treatment of advanced melanoma in adult patients. T-VEC is engineered with the deletions of the neurovirulence gene *RL1* which encodes *ICP34.5*, and the deletion of *ICP47*. Additionally, the granulocyte–macrophage colony-stimulating factor (GM-CSF) promoter is inserted into T-VEC[7]. A similar oncolytic virus, HSV1716, has been proven to be safe in pediatric patients following a phase I clinical trial for patients with relapsed non-CNS solid tumors[8]. HSV1716, an HSV-1 strain 17 derivative, contains the deletion of *RL1* gene encoding ICP34.5[8]. While the clinical trial did demonstrate the safety of a single intratumoral injection and that the virotherapy was well-tolerated by the patients, no objective responses were seen[8]. Conclusions from this study suggest that oncolytic viruses as a single-agent treatment may not be effective for pediatric cancer, and combination treatments with other immunotherapeutics or chemotherapies should be investigated.

This thesis explores the use of a combination treatment of the oncolytic virus, HSV1716 with the chemotherapy agent, trabectedin. Originally isolated from a marine source, trabectedin is a DNA binder that activates caspase-8-dependent apoptosis in myelomonocytic cells[9]. Additionally, it has been shown to deplete MDSCs and TAMs, which is a key component of trabectedin's anti-tumor efficacy[9]. In a 2018 study, the Cripe Lab combined trabectedin with the oHSV, rRp450. This study was conducted in two Ewing sarcoma Patient Derived Xenograft,

PDX, models, and the combination of trabectedin and oHSV led to tumor regression in 78% of the treatment group[10]. However, it is unclear if this combination myelolytic-virotherapy therapy will produce similar results in non-Ewing pediatric solid tumors, and studies to test this treatment's generalizability are ongoing.

Osteosarcoma is the most common form of pediatric bone cancer, and this disease typically occurs in the long bones such as the femur or tibia. Current treatments for localized disease have a 5- year survival rate of 78%[11]. Once the disease has metastasized, it often travels to the lungs. The survival rate for metastatic disease is only between 10-30%[12]. This poor prognosis following metastasis requires new treatment options to be made available. However, difficulties with treating osteosarcomas include that they are poorly immunogenic tumors due to a lack of tumor neo-antigens for targeting and low levels of CD8 infiltrate in the microenvironment. As such, they are characterized as cold tumors, a feature that is common among pediatric cancers[11]. One study demonstrated that human osteosarcoma xenograft mouse models responded to two intratumoral injections of the oHSV, rRp450[13]. Given that osteosarcomas have demonstrated previous sensitivity to oHSV, osteosarcoma became one of the targets for determining if the combination therapy of oHSV and trabectedin is generalizable to other models outside of Ewing sarcoma.

In order to determine the generalizability of the combination therapy, murine tumor models of osteosarcoma were studied in immunocompetent mice. Unlike the studies seen in the PDX models, the treatment in murine, immunocompetent models produced extreme toxicity. This toxicity included rapid weight loss and respiratory distress. While the combination treatment remains promising, the focus of my research has shifted from assessing the generalizability of the treatment to mitigating the combination treatment's toxicity while

maintaining its efficacy. As such, this thesis details the studies performed to control the toxicity of the combination therapy of the oHSV, HSV1716, and the chemotherapy agent, trabectedin.

**Preliminary Data**

*Survival Study of F420 implanted Immunocompetent Mice Treated with HSV1716 and Trabectedin*

The first study of the combination therapy in immunocompetent mice was with the murine osteosarcoma model, F420. Figure 1. shows a schematic of the treatment regimen. To summarize, the mice were injected subcutaneously with $5.0x10^6$ F420 cells. Once the tumors reached approximately 150 to 200 $mm^3$, the treatment began. The mice were divided into four study groups: PBS alone injected intratumorally on day 0, 2, and 4, HSV1716 injected intratumorally on day 0, 2, and 4, Trabectedin alone administered intravenously via the tail vein on day 0 and 7, and the combination treatment of trabectedin administered intravenously on day 0 and 7 with HSV1716 being injected intratumorally on day 0, 2, and 4. HSV1716 was administered at a concentration of $1.0x10^8$ pfu, and trabectedin was administered at a dose of 0.15mg/kg. The tumors were then measured twice a week with calipers. Once the mice reached the endpoint criteria which included either excess tumor burden (tumor size greater than $2000mm^3$), tail necrosis, or a 20% reduction in body weight, the mice were euthanized. To note, trabectedin is vesicant that can cause tail necrosis if it comes in contact with the skin. As such, tail blistering is common if the drug leaks during the tail vein injection.

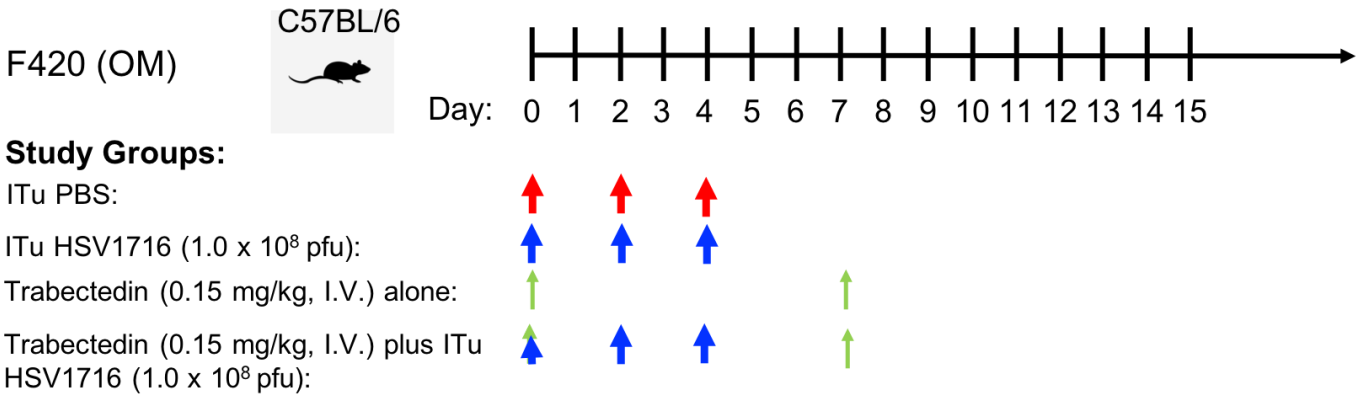


**Figure 1.** Treatment Schematic for Survival Studies Involving the Combination Therapy of Trabectedin and HSV1716.

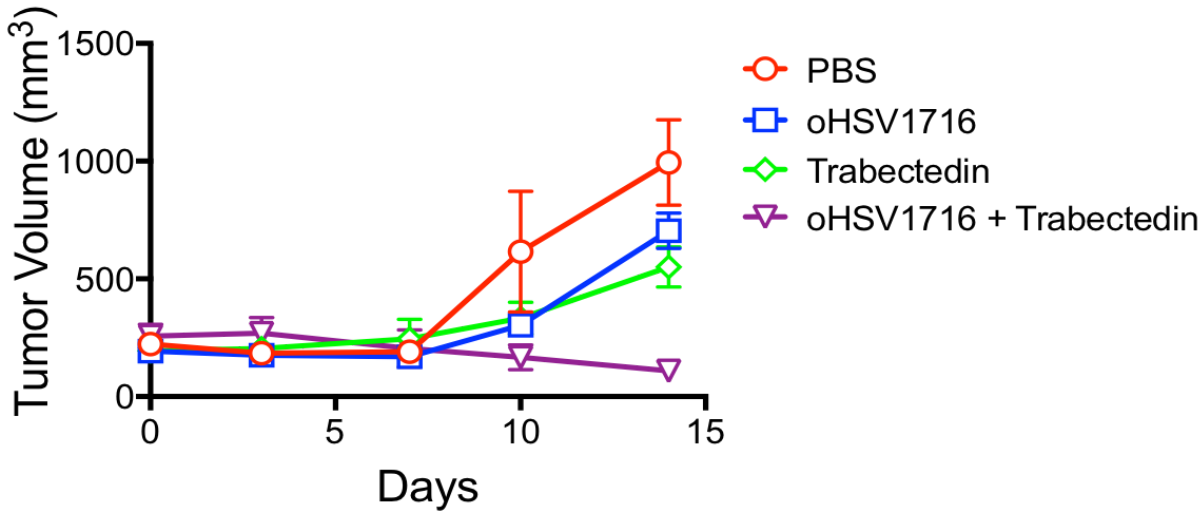


**Figure 2.** Average Tumor Volume Progression Day 0 through Day 14. The combination treatment is able to control tumor growth better than either of the single agent treatments, trabectedin alone or HSV1716 alone.

Figure 2. shows the tumor progression data from the survival study. This figure demonstrates that the combination of trabectedin with HSV1716 is able to control tumor growth better than either HSV1716 or trabectedin alone at early time points in the survival study. This suggests that the combination treatment does demonstrate efficacy. However, of the nine mice in

the combination group, six of the mice were either found dead or had to be euthanized due to toxicity. For the mice that had to be euthanized, increased respiratory rate and loss of body weight were symptomatic of the toxicity. Comparatively, only two of the nine mice treated with trabectedin alone succumbed to toxicity, and zero of the ten mice treated with HSV1716 alone had to be euthanized due to toxicity. Figure 3 shows the body weights of each mouse during the study. Table 1 provides a summary of the cause for study termination for each cohort.

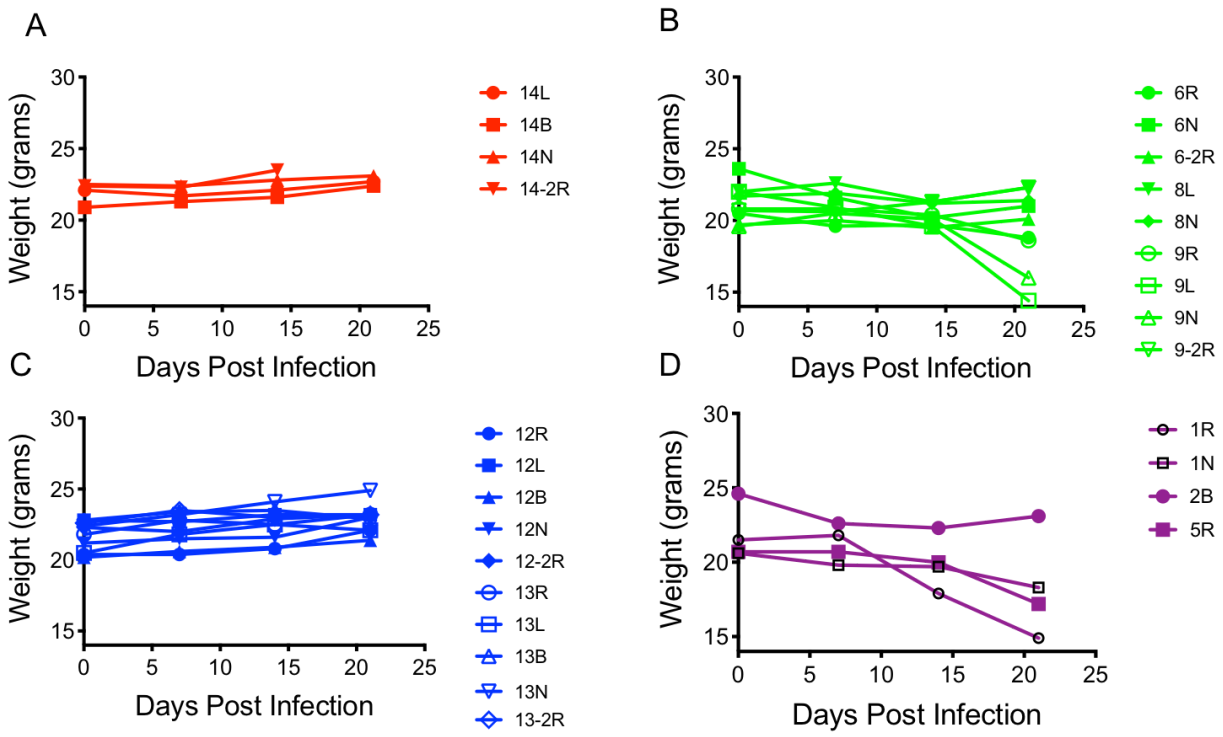


**Figure 3.** Body Weights of Mice. A. PBS B. Trabectedin alone C. HSV1716 D. Combination Trabectedin and HSV1716.

| Group | n | Toxicity | Tail Necrosis | Tumor Burden | Termination of Study |
|---|---|---|---|---|---|
| Trabectedin & HSV1716 | 9 | 6 | 1 | 1 | 1 |
| Trabectedin | 9 | 2 | 3 | 4 | 0 |
| HSV1716 | 10 | 0 | 0 | 9 | 1 |

**Table 1.** Summary of F420 survival study cause of euthanasia/death for each study cohort.

*Reduction of Trabectedin Dose Levels to Reduce Toxicity*

In an effort to address the toxicity, researchers in the Cripe lab performed a dose de-escalation study with the trabectedin being reduced from 0.15 mg/kg to 0.075 mg/kg, 0.0375 mg/kg, 0.0188 mg/kg, 0.0094 mg/kg, and 0.0047 mg/kg. The mice of this study were divided into five groups for each dose of trabectedin, and then administered the combination HSV1716 and trabectedin treatment according to the treatment schedule outlined in Figure 1. The concentration of HSV1716 remained the same as the initial study at $1.0x10^8$ pfu. Figure 4 shows the tumor volume progression data for each mice cohort. By day 14, none of the cohorts demonstrated the ability to either reduce tumor volume or prevent further tumor enlargement. However, no mice in any of the groups succumbed to toxicity. As such, at all doses of trabectedin at and below 0.075 mg/kg, it can be concluded that both the efficacy and the toxicity of the combination treatment was eliminated.

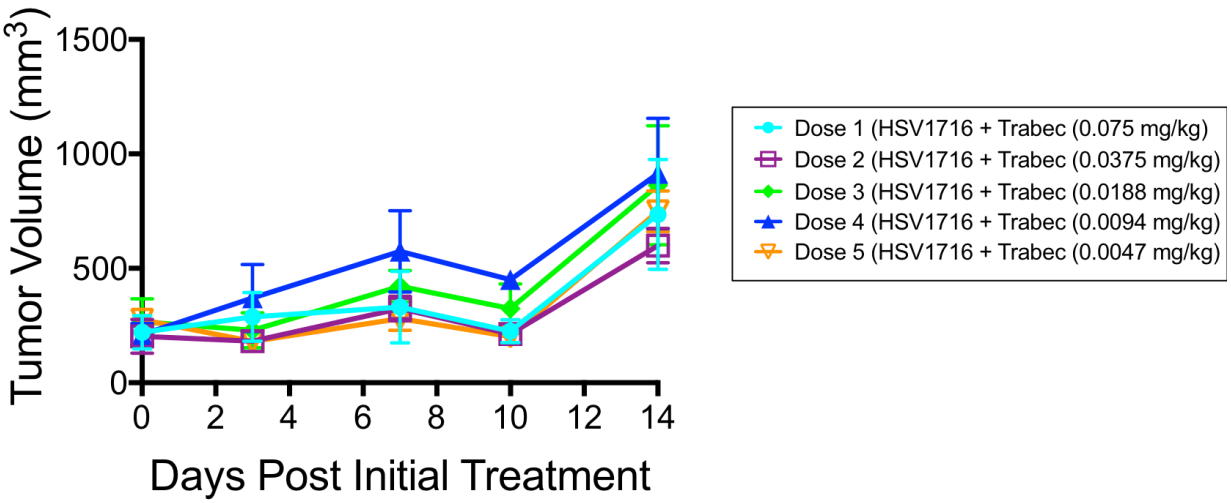


**Figure 4.** Average tumor volume progression for trabectedin dose de-escalation study. At all doses of trabectedin used, the efficacy of the combination treatment is eliminated. Tumor volume is neither reduced, nor held constant by day 14.

*Reduction in Number of Trabectedin Doses to Reduce Toxicity*

The next experiment performed by Cripe lab researchers was to use the initial dose of trabectedin (0.15 mg/kg) with the number of doses being administered reduced from two administered on day 0 and 7, to just one dose administered on day 0. The dosing regimen for HSV1716 ($1.0x10^8$ pfu) remained the same as the one outlined in Figure 1. In this survival study, the trabectedin alone group, like the combination treatment group, was only administered one dose of trabectedin on day 0. Figure 5 depicts the tumor volume progression data for each of the four cohorts (PBS alone, HSV1716 alone, trabectedin alone, and combination HSV1716 with trabectedin). In figure 5, the tumor volume progression indicates that the combination treatment is able to control tumor volume progression slightly better than the trabectedin alone group at early time points. However, Figure 6 demonstrates that the percent survival of the trabectedin alone group and the combination group remained similar. As such, it can be concluded that the elimination of the second dose of trabectedin limited the efficacy of the combination treatment. Additionally, of the thirteen mice in the trabectedin alone group, two had to be euthanized due to

toxicity. Then, in the combination treatment group seven of the fourteen mice had to be euthanized due to toxicity. Therefore, elimination of the second dose did not significantly improve the toxicity of the combination treatment. Table 2 provides a summary of the cause for study termination for each cohort.

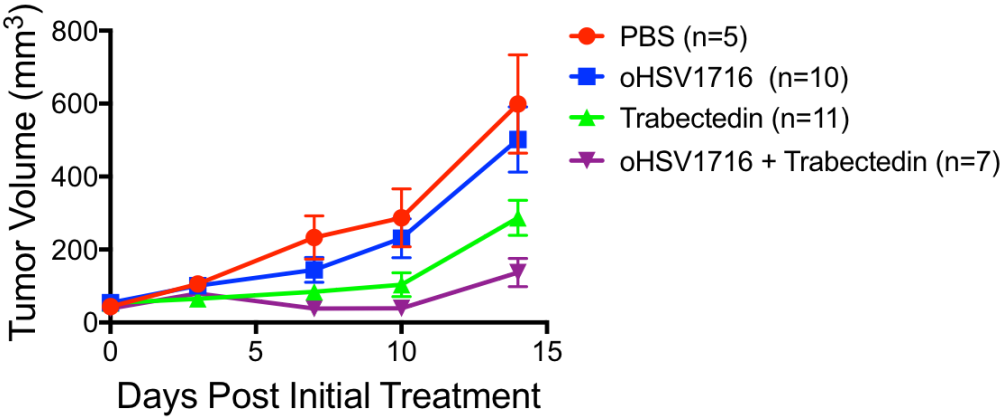


**Figure 5.** Average tumor volume progression for trabectedin dose elimination study. Combination treatment of trabectedin and HSV1716 has a slight ability to control tumor volume progression compared to PBS, HSV1716 alone, and trabectedin.

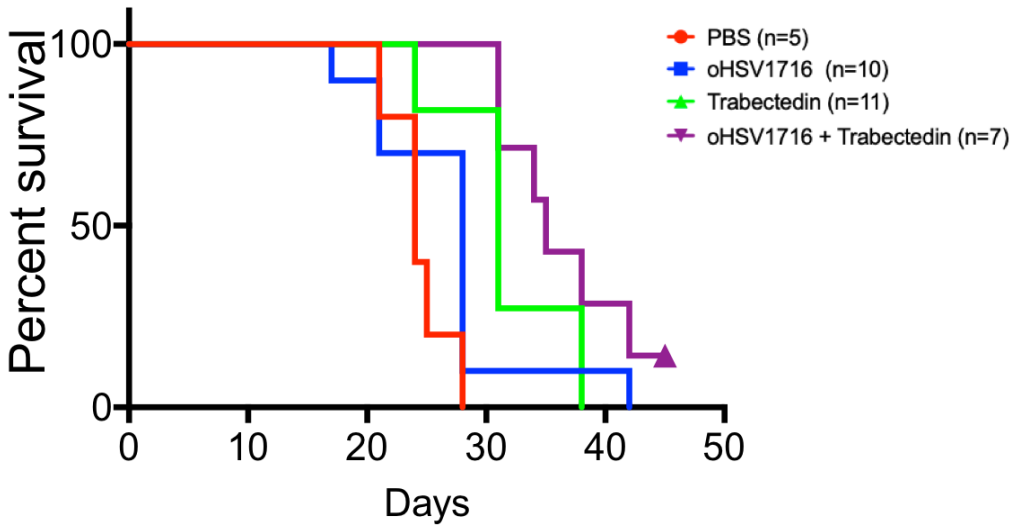


**Figure 6.** Percent survival for trabectedin dose elimination study. The combination HSV1716 and trabectedin did not prologue survival. This graph is suggestive of a loss of efficacy in the combination group.

| Group | n | Toxicity | Tail Necrosis | Tumor Burden | Termination of Study |
|---|---|---|---|---|---|
| Trabectedin & HSV1716 | 14 | 7 | 0 | 6 | 1 |
| Trabectedin | 13 | 2 | 0 | 11 | 0 |
| HSV1716 | 10 | 0 | 0 | 10 | 0 |

**Table 2.** Summary of trabectedin dose elimination survival study cause of euthanasia/ death for each study cohort.

*Evaluation of the Effect of T Cells on Tumor Response*

The final preliminary experiment was to evaluate the effect of T cells on the myleloytic virotherapy by injecting F420 cells on the flank of athymic nude mice and following the treatment schedule outlined in figure 1. Figure 7 shows the average tumor volume progression data for each of the four cohorts, and HSV1716 alone, trabectedin alone, and the combination treatment of HSV1716 and trabectedin exhibit similar trends. As such, it can be concluded that

the tumor response is limited in the athymic nude strain. However, weight loss due to toxicity is also absent in the athymic nude mice. Figure 8 compares the body weights of mice receiving the same treatment regimen outlined in Figure 1 in C57BL/6 mice and athymic (nu/nu) mice. This figure demonstrates that toxicity due to weight loss correlates with either T cell presence or mouse strain.

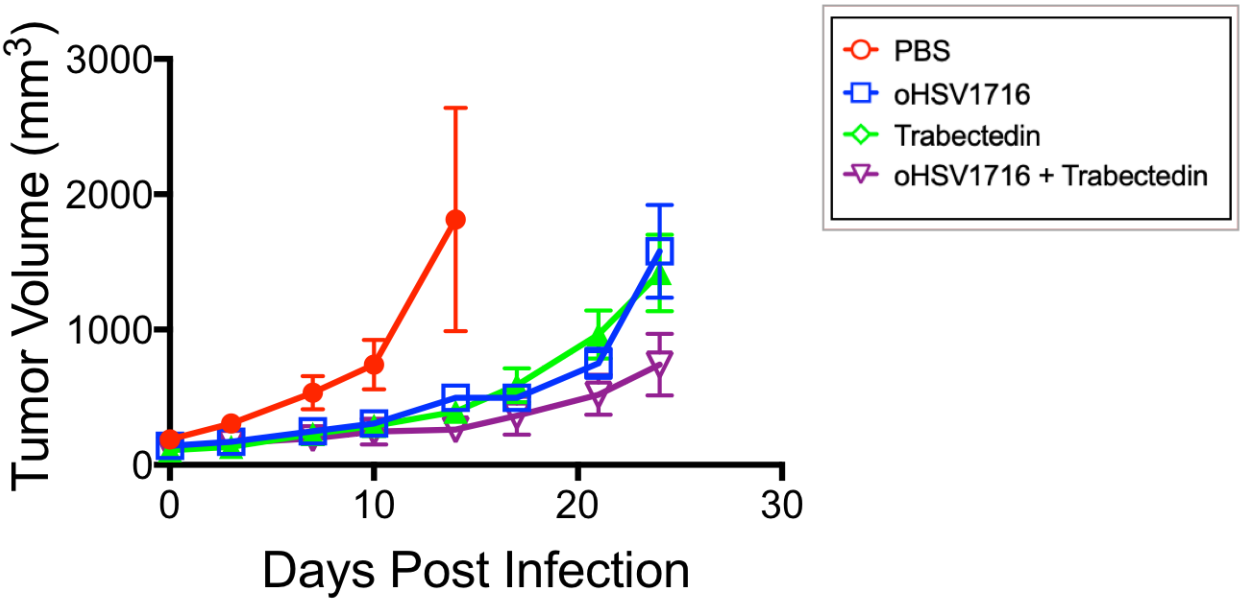


**Figure 7.** Average tumor volume progression for athymic nude study. Tumor volume progression is similar among HSV1716 alone, trabectedin alone, and combination trabectedin and HSV1716. This is suggestive that the tumor response of the combination treatment is lost athymic nude mice.

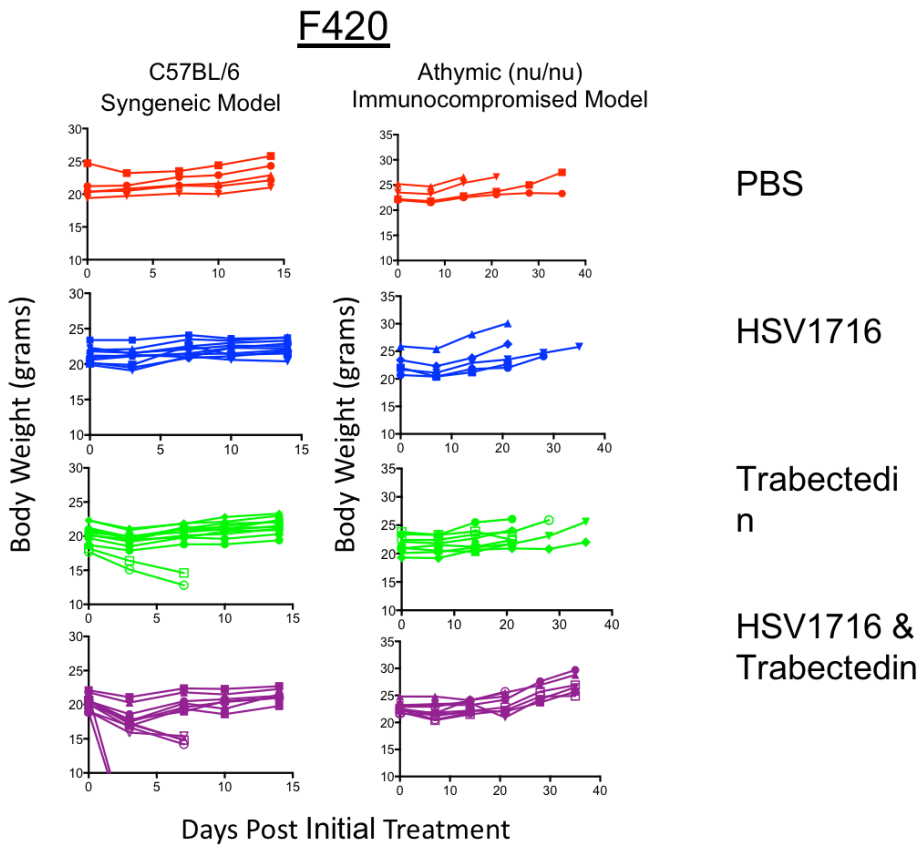


**Figure 8.** Weight comparison between treatment groups in C57BL/6 mice and athymic (nu/nu) mice. Toxicity is lost in the athymic mice suggessting that weight loss from toxicity correlates with T cell presence or mouse strain.

**Conclusions from Preliminary Data**

From the experiments and results outlined above, the Cripe lab researchers came to several conclusions. First, reducing the amount of trabectedin delivered either by reducing the dose amount or by eliminating the second dose did not lead to adequate tumor response. Furthermore, the toxicity was only controlled when the dose amount was reduced. In this case, toxicity was controlled, yet efficacy was lost. However, the second dose elimination study exhibited a loss of efficacy while maintaining toxicity. As such, it seems critical that both doses of trabectedin be administered for maintaining the efficacy of the treatment, and that the therapeutic window of trabectedin is greater than 0.075 mg/kg. Then from the athymic nude

study it can be concluded that treatment efficacy and toxicity seem to correlate with either T cells or mouse strain.

**Introduction to Thesis Experiments**

Despite the challenges with managing the toxicity and efficacy of the combination treatment, we continued to pursue the treatment due to the promising results seen in Ewing sarcoma[10]. However, instead of focusing on the generalizability of the combination treatment, we instead focused on finding a way to mitigate the toxicity of the combination treatment in the immunocompetent model while maintaining efficacious tumor responses. To do this, we first sought to determine the mechanism of the toxicity. After consulting with Dr. Timothy Cripe MD, PhD, and PI of the lab, two mechanisms were proposed for the toxicity including tumor lysis syndrome and cytokine release syndrome.

When tumor cells lyse, they release potassium, phosphorus, and nucleic acids into the bloodstream which can lead to electrolyte and metabolic disturbances[14]. Diagnosis of laboratory tumor lysis syndrome includes the presence of two or more of the following metabolic abnormalities: hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia. Additionally, the onset of these metabolic disturbances must occur three to seven days following treatment initiation. Clinically, these disturbances are associated with toxic side effects such as death due to multiorgan failure[14].

Cytokine release syndrome is a systemic inflammatory response that can be caused by both infection and drug treatment. Like tumor lysis syndrome, it can lead to multiorgan failure and death. Notably, cytokine release syndrome is among the most frequent adverse events that can occur following t-cell engaging immunotherapy[15]. The most commonly elevated cytokines in people experiencing cytokine release syndrome include IL-6, IL-10, and IFNγ. Additionally, in

murine models of cytokine release syndrome, IL-6 signaling pathways have been implicated in directing the pathophysiology of the syndrome. As such, blocking IL-6 signaling pathways via monoclonal antibody therapy is a treatment for cytokine release syndrome that can be applied in severe cases.

The first set of experiments for the thesis included determining whether tumor lysis syndrome or cytokine release syndrome was occurring in mice treated with the combination treatment. Then, based upon those study results, we then completed survival studies with additional therapeutics. Our final experiment in the study involved reducing the dose of trabectedin again to determine the therapeutic window in which the treatment toxicity was minimized while maintaining effective tumor responses.

**Thesis Experiments**

*Determining if Tumor Lysis Syndrome or Cytokine Release Syndrome is occurring in Mice Treated with the Combination Therapy of HSV1716 and Trabectedin*

To determine if the mice bearing F420 tumors treated with the combination treatment were experiencing tumor lysis syndrome, we sent blood serum to the hospital laboratory at Nationwide Children's Hospital to complete a metabolic panel. Prior to sending the serum, we administered one dose of trabectedin at 0.15 mg/kg, and the mice either received HSV1716 according to the dosing regimen in Figure 1, or they only received the single trabectedin dose. Then, on day 7 after the single trabectedin dose was administered, the mice were euthanized and their blood was collected via retro-orbital bleeding. The blood samples were centrifuged to separate the serum, and then the serum was sent for analysis. The metabolic panel measured albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, blood urine nitrogen (BUN), calcium, creatinine, lactate dehydrogenase (LDH), sodium, potassium,

chloride, carbon dioxide, total protein, and uric acid. Figure 9 shows the results from the metabolic panel. If tumor lysis syndrome was occurring, we anticipated seeing elevated potassium and phosphorus with low calcium. None of these were far outside of the normal range described in the literature for C57BL/6 albino mice. This suggests that tumor lysis syndrome is not the mechanism for causing the toxicity, However, both ALT and AST enzymes were elevated suggesting evidence of hepatotoxicity.

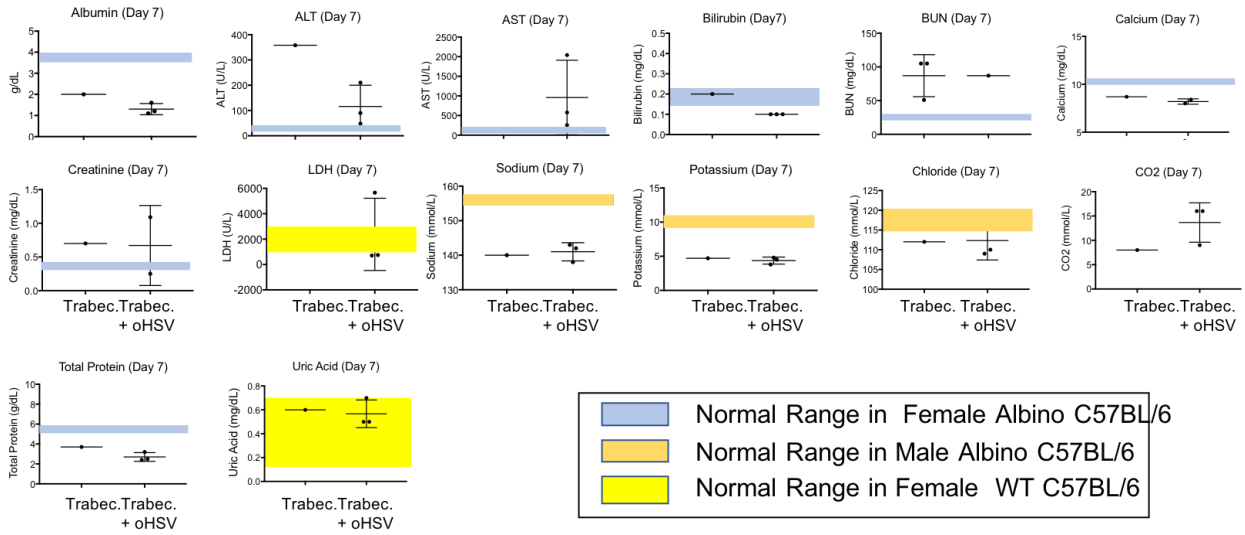


**Figure 9.** Metabolic panel of C57BL/6 mice treated with a single dose of trabectedin (0.15 mg/kg) with and without HSV1716 treatment. The serum was collected from the mice on day 7 of treatment. High ALT and AST enzymes indicates hepatotoxicity.

We then completed a mouse-specific cytokine array panel using serum from the same four mice that we also used for the metabolic panel. As such, the mice either received one dose of trabectedin and HSV1716 or just one dose of trabectedin alone. Additionally, the cytokine array panel shows only four samples as that is the maximum number that can be tested with one kit. We chose to use two combination treatment samples, one trabectedin alone sample, and one control sample. Figure 10 shows the results from the cytokine array panel. The only cytokine that

was present in the combination treatment samples, but absent from both the control and trabectedin alone samples was granulocyte colony-stimulating factor (G-CSF).

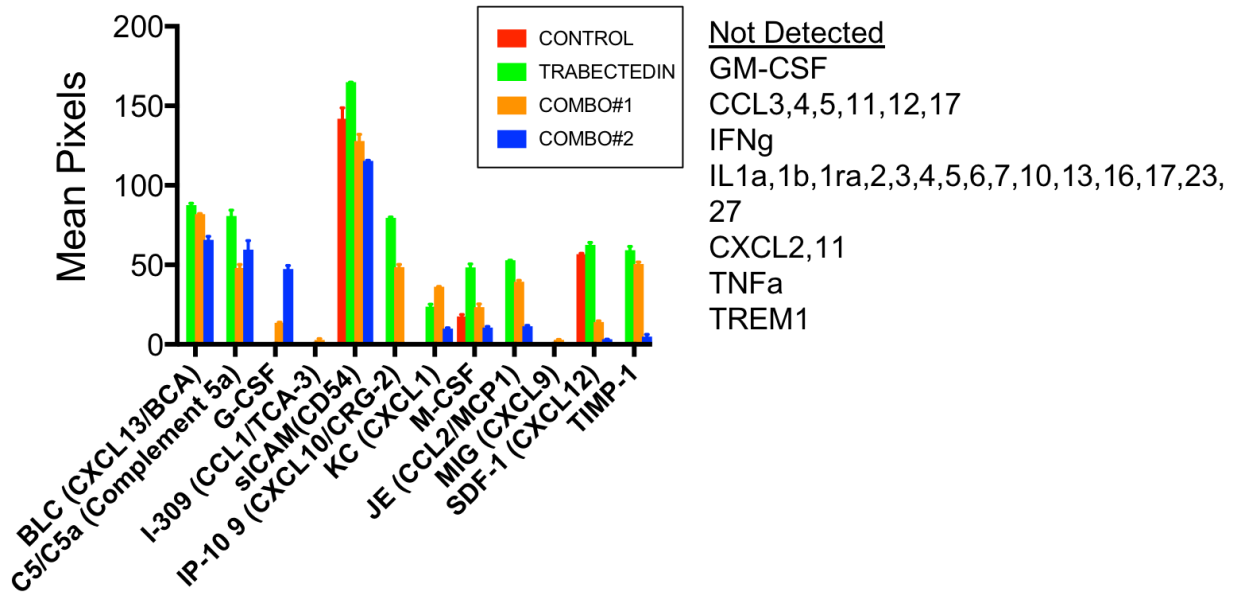


**Figure 10.** Cytokine array panel. Samples from a control mouse, a mouse that received trabectedin alone, and two combination trabectedin and HSV1716 mice. G-CSF is the only cytokine that is present exclusively in the combination therapy mice.

G-CSF is a pro-inflammatory cytokine that induces the mobilization of neutrophils from bone marrow to the periphery. The role of G-CSF in solid tumors and in the tumor microenvironment is limited. However, one group out of the University of New Mexico studied Anti G-CSF treatment in colorectal cancers[17]. Interestingly, they found that anti G-CSF treatment led to a reduction of IL-10 and an increase in IFNγ. As noted in the introduction of the paper, M2-like macrophages are protumorigenic and are polarized to the M2 phenotype by IL-10 whereas M1-like macrophages are polarized by IFNγ. As such, anti G-CSF treatment could influence macrophage polarization from protumorigenic to anti- tumorigenic. Additionally, if G-CSF was playing a role in the toxicity of the combination treatment, reduction of G-CSF by treating the mice with anti G-CSF antibodies would lead to a reduction in the toxicity.

*Evaluating Anti G-CSF Treatment on Reducing the Toxicity of the Combination Treatment of HSV1716 with Trabectedin*

To evaluate the effect of anti G-CSF antibody treatment in mice injected with F420 tumors, we completed a pilot study. Ten mice were injected subcutaneously with $5.0x10^6$ F420 cells. Once the tumors reached 150 $mm^2$ - 200 $mm^2$ treatment began. As with previous studies, trabectedin was administered on day 0 and day 7 at a dose of 0.15 mg/kg via the tail vein and HSV1716 was administered on day 0, day 2, and day 4 intratumorally at a dose of $1.0x10^8$ pfu. Anti G-CSF antibody injected intraperitoneally at a dose of 25µg dissolved in 100µL of PBS. Anti G-CSF was administered on day 0, day 2, and day 4. Figure 11 shows the tumor volume progression for each mouse in the study. Of note, one mouse was found dead on day 7 of the experiment, and one mouse had to be euthanized on day 10 due to low body weight. Therefore, the anti G-CSF treatment was not successful at preventing toxicity.

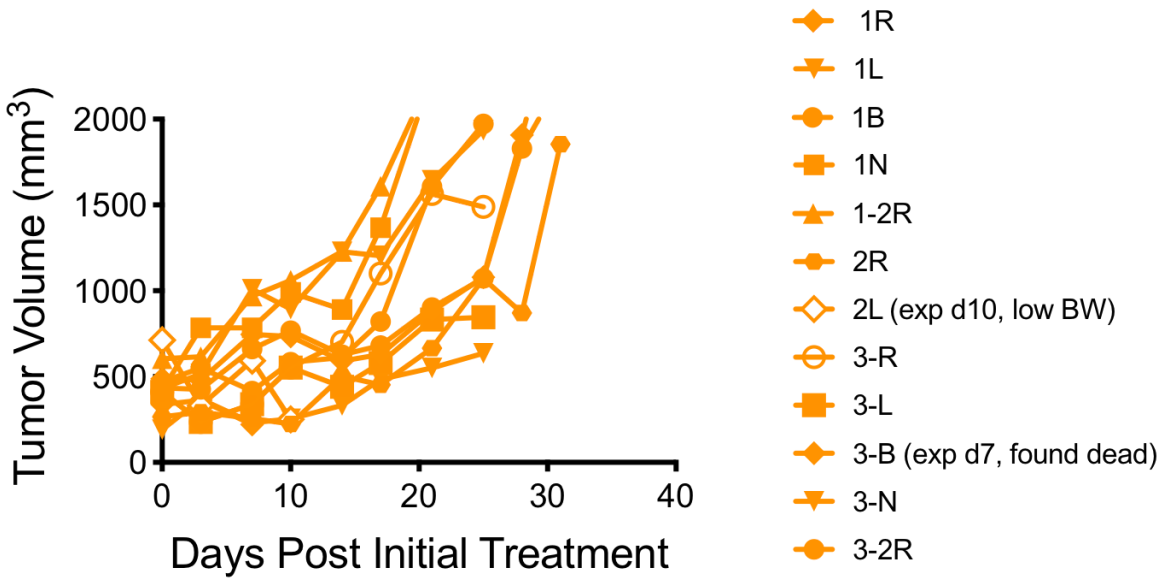


**Figure 11.** Tumor volume progression for mice treated with combination treatment of trabectedin and HSV1716 and with anti G-CSF antibodies. Two mice, 2L and 3B, were either found dead or had to be euthanized due to toxicity. All other mice were euthanized due to excessive tumor burden.

Figure 12 shows the anti G-CSF cohort in comparison to cohorts of mice in previous studies receiving trabectedin alone, HSV1716 alone, and the combination treatment of HSV1716 and trabectedin. The anti G-CSF cohort was not able to control or reduce tumor growth as well as the combination trabectedin and HSV1716 group. As such, the addition of anti G-CSF therapy neither improved toxicity or maintained the efficacy of the combination treatment.

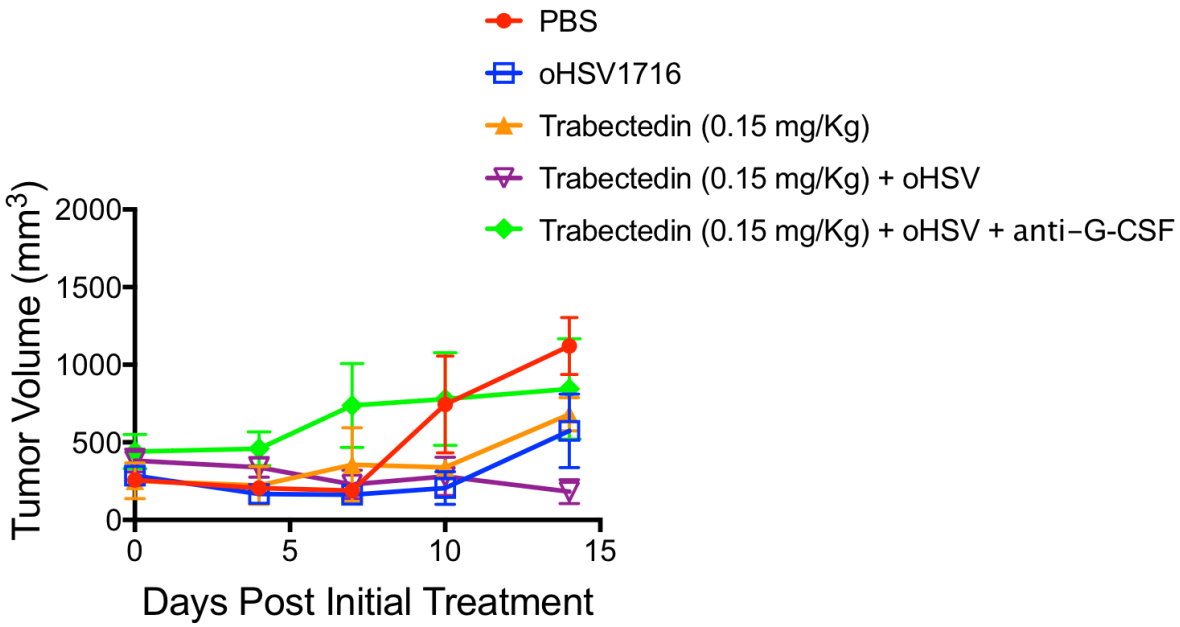


**Figure 12.** Comparison of combination treatment (trabectedin, HSV1716, and anti G-CSF) with previous treatment cohorts. The combination of the three therapeutics did not reduce or halt tumor growth. The anti G-CSF treatment reduced the efficacy of the combination treatment of trabectedin and HSV1716.

*Combining Dexamethasone with the Combination of Trabectedin and HSV1716 to Control Toxicity*

Following the negative results from the anti G-CSF study, we turned to the literature to reexamine if any groups had success controlling the toxicity of trabectedin alone. We found one group that utilized dexamethasone as a pretreatment prior to the administration of trabectedin to ameliorate the drug-induced hepatotoxicity (High-Dose Dexamethasone). Dexamethasone is a steroid commonly used to treat inflammation. The study authors concluded that a single high-

dose pretreatment with dexamethasone was sufficient to control toxicity without limiting the efficacy of the treatment[18]. However, this study was predominantly completed in the Wistar rat.

Based upon the promising results in the literature, we completed a survival study with a dexamethasone pretreatment to examine its ability to reduce the toxicity in the combination treatment in the F420 model. Mice were injected subcutaneously with $5.0x10^6$ F420 cells. Once the tumors reached 150 $mm^2$ - 200 $mm^2$ treatment began. Figure 13 provides a schematic of the treatment regimen. To summarize, 48 hours prior to the first treatment with trabectedin and HSV1716, all mice were treated with 40 mg/kg of dexamethasone via an intraperitoneal injection. Then, 24 hours prior to treatment all mice received a second pretreatment of the dexamethasone (40 mg/kg delivered via an intraperitoneal injection). On day 0, the mice were divided into their respective cohorts: PBS, HSV1716 ($1.0x10^8$ pfu), trabectedin (0.15 mg/kg), and combination HSV1716 and trabectedin. HSV1716 was administered intratumorally on day 0, day 2, and day 4. Trabectedin was administered on day 0 and day 7. The mice were then measured with calipers twice a week to measure tumor growth.

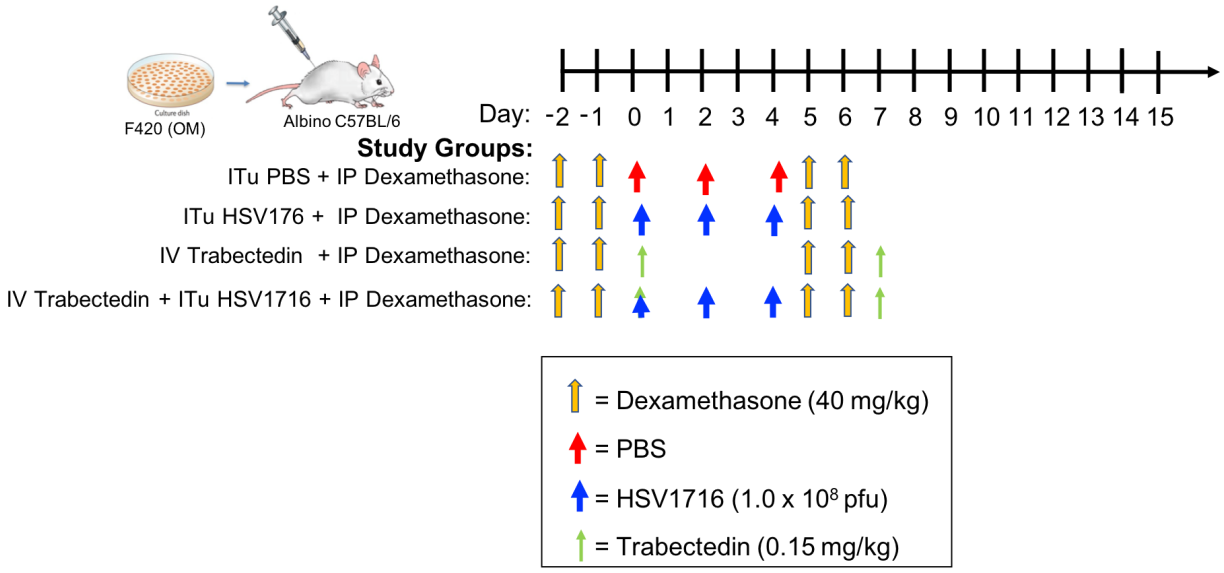


**Figure 13.** Treatment schematic for survival study with dexamethasone.

Of the fourteen mice in the combination group, four mice were either euthanized or found dead due to the toxicity of the treatment. As such, the toxicity of the treatment was not significantly reduced with the pretreatment with the dexamethasone. Figure 14 shows the percent survival of the four cohorts, and the combination treatment was not more efficacious. Ultimately, we concluded that dexamethasone did not markedly reduce the toxicity and it limited the combination treatments efficacy rendering it an ineffective treatment addition.

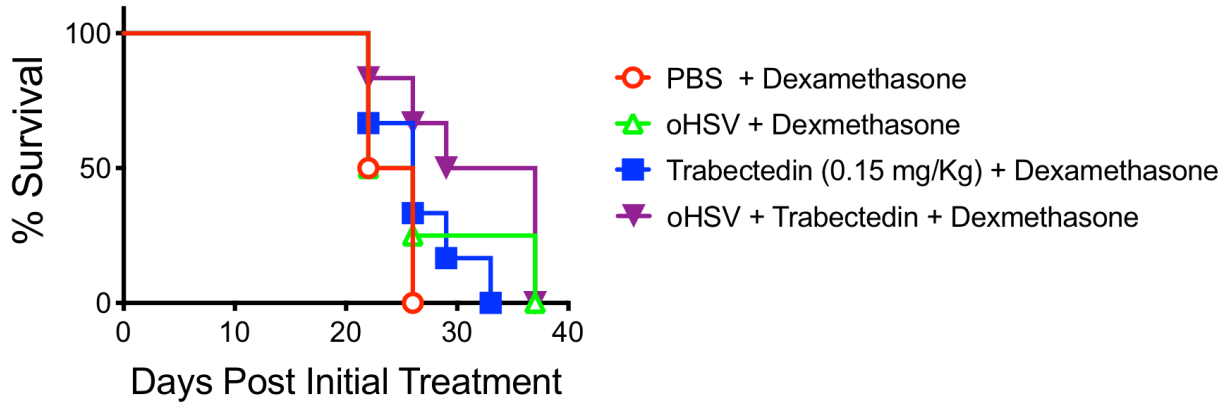


**Figure 14.** Percent survival of cohorts in the dexamethasone survival study. The combination treatment + dexamethasone demonstrates reduced efficacy compared to the combination treatment alone.

*Exploring the Therapeutic Window of Trabectedin in a Second Dose De-escalation Study*

With negative results from both anti G-CSF treatment and dexamethasone pretreatment, we returned to evaluating the therapeutic window of trabectedin. The therapeutic window refers to the dose concentration at which the drug is effective but has limited toxicity. Going too far below the therapeutic window will result in a loss of efficacy whereas going above the therapeutic window will lead to extreme toxicity. Previous studies have reported that the therapeutic window of trabectedin is narrow, so we decided to only drop the dose of trabectedin from 0.15 mg/kg to 0.125 mg/kg and 0.10 mg/kg[16]. In contrast, our first dose de-escalation study reduced the dose of trabectedin in half and then continued to decrease the dose.

In accordance with our previous animal studies, we began by injecting mice subcutaneously with $5.0x10^6$ F420 cells. Once the tumors reached 150 $mm^2$ - 200 $mm^2$ treatment began. The mice were divided into two cohorts: the combination of HSV1716 ($1.0x10^8$ pfu) with trabectedin (0.125 mg/kg) and the combination of HSV1716 ($1.0x10^8$ pfu) with trabectedin (0.10 mg/kg). The dosing regimen remained the same with HSV1716 being administered intratumorally on day 0, day 2, and day 4. Trabectedin was delivered via the tail vein on day 0 and day 7. This study is currently ongoing at the time of submitting this thesis, so completed data is not available to determine if either of the trabectedin dose groups produced similar efficacy to the standard 0.15 mg/kg treatment we had been using for our studies. However, of the 26 mice in the study, 5 mice had to be euthanized due to weight loss associated with toxicity. Therefore, further investigation into managing the toxicity of the combination treatment is warranted.

**Conclusions and Future Directions**

After completing several experiments to mitigate the toxicity of the combination treatment, we have remained unsuccessful in both controlling the toxicity while simultaneously maintaining efficacy. From our metabolic panel, we were able to eliminate both tumor lysis syndrome and cytokine release syndrome as causes of the treatment toxicity. However, our cytokine array panel indicated that G-CSF may be playing a role in the toxicity seen in the combination treatment, anti G-CSF antibody therapy did not eliminate the toxicity. Additionally, this treatment limited the efficacy of the combination treatment. Then, we used dexamethasone in an attempt to control the toxicity. Similar to the ant G-CSF study, pretreatment with dexamethasone did not lead to a significant reduction in toxicity, and it limited the efficacy of the combination treatment. Finally, we returned to examining the therapeutic window of the trabectedin by lowering the dose from 0.15 mg/kg to 0.125 mg/kg and 0.10 mg/kg. This study is

currently ongoing, but early analysis indicates that reducing the trabectedin dose still leads to toxicity in the combination treatment.

Our future directions include further examining the hepatotoxicity that was indicated by the metabolic panel with the high ALT and AST enzyme levels. We recently injected F420 tumor cells in mice to begin a large scale metabolic panel study. In this study, mice will be divided into the following cohorts: PBS, HSV1716 alone, trabectedin alone, and the combination of trabectedin and HSV1716. Mice from each cohort will either be euthanized on day 3 or day 7 following the initiation of treatment and blood will be collected for serum analysis. The goal of this study is to confirm that the toxicity of the combination treatment is caused by damage to the liver.

The next study we would like to complete is to examine if the toxicity is strain specific. In our preliminary data, we demonstrated that the toxicity (and efficacy) of the combination treatment was eliminated in the athymic nude mouse model. However, it is difficult to determine from this study if the lack of toxicity is T-cell dependent or strain dependent. We will determine if the toxicity is strain specific for C57BL/6 mice by comparing this mouse model to another immunocompetent mouse background, balb/c mice. In this study, we will utilize 4T1 cells, a mammary carcinoma, as F420 cells cannot be grown in balb/c mice. We will complete a survival study with the four cohorts: PBS, HSV1716, trabectedin, and the combination of trabectedin and HSV1716. If the toxicity is reduced in the balb/c model, it would confirm that the toxicity of the combination therapy is C57BL/6 strain dependent.

Finally, future directions for this project include substituting trabectedin for a recently approved analog, lurbinectedin. Similar to trabectedin, lurbinectedin is a DNA minor-groove binder that is thought to affect the tumor microenvironment by depleting macrophages[19]. Early

studies on lurbinectedin suggest it may be less toxic than trabectedin[19]. As such, this warrants us to complete a study in which the combination of trabectedin and HSV1716 is compared to the combination treatment of lurbinectedin and HSV1716.

Several limitations may have influenced our study results. One important consideration is that oncolytic viruses have higher replication levels in human cells. As such, a reduction of viral replication in the murine models could lead to diminishing efficacy. This would explain the reduced efficacy we have seen in murine models. Ultimately, there still remains a need for the development of treatments for pediatric solid tumors, especially in case of relapsed or malignant disease. Oncolytic viruses in combination with other therapeutics, such as trabectedin, need to be investigated further to determine if they can be developed into viable treatment options.

**Materials and Methods**

**Cell Lines and Virus.** For the syngeneic studies, we used the F420 osteosarcoma cell line. All cell lines identities were confirmed by short tandem repeat genotyping, and mycoplasma testing. We used HSV1716 as the oHSV virus (delivered at 1.0 x $10^8$ pfu).

**Compounds and Reagents.** Trabectedin is provided by Janssen Pharmaceutical Companies of Johnson and Johnson.

**Animal Studies.** All animal studies were approved by the Institutional Animal Care and Use Committee for the Abigail Wexner Research Institute at Nationwide Children's Hospital. C57BL/6 and C57BL/6 albino mice were used in the studies.

***In Vivo* Efficacy Studies.** Tumors were implanted subcutaneously on the flank of the mice. Once tumors reach a mean volume between 150 $mm^2$ and 250 $mm^2$, the mice were randomized into four study groups: control (PBS), HSV1716 alone, trabectedin alone, and the combination of trabectedin and HSV1716. Trabectedin was administered via a tail vein injection, and HSV1716 was delivered intratumorally. Virus was given on day 0, day 2, and day 4. Trabectedin was given on day 0 and day 7 (Studies that deviated from this general scheme have detailed descriptions of how the treatment was changed under their appropriate study analysis). The mice were followed until the animals reached endpoint criteria (tumor volume >2000$mm^2$, unusual mouse behavior, lack of movement, poor posture, or body weight loss >20%) or 60 days. During the study, tumor size was measured using calipers twice each week. Tumor volume is calculated by the formula: (length of tumor) x (width of tumor)$^2$ x ($\pi$/6). Mice were humanely euthanized by $CO_2$ asphyxiation and cervical dislocation once endpoint criteria was met.

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